techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding UGGT. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2'O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept can be extended by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

**N. <u>High-Throughput Screening</u>**

The power of high throughput screening is utilized in the search for new compounds (in addition to thapsigargin) which are capable of mobilizing mis-folded or incompletely assembled proteins from the ER, thus enabling their surface delivery. The following protocol is designed to permit rapid automated screening of large numbers of compounds useful for practicing the claimed invention. The demonstration that thapsigargin produces a positive result when tested in the high-throughput screening assays will act as a positive control. For general information on high-throughput screening, see, for example, <u>Cost-Effective Strategies for Automated and Accelerated High-Throughput Screening</u>, IBCS Biomedical Library Series, IBC United States Conferences (February, 1996); John P. Devlin (Editor), <u>High Throughput Screening</u>, Marcel Kedder (1998); U.S. Patent No. 5,763, 263;

**CTL-Mediated Cell Lysis.** Cytotoxic T cells recognize their targets through interactions with Major Histocompatibility Complex (MHC) class I proteins expressed on the target cell surfaces. MHC class I is a complex composed of the MHC class I heavy chain (a transmembrane protein) and β2-microglobulin (β2m). MHC class I heavy chains assemble with β2m during their post-synthetic residence in the ER. Each MHC class I heavy chain also binds to a peptide produced by cytosolic proteolysis catalyzed by the proteasome and transported into the lumen of the ER by the ATP-dependent transporter

associated with antigen processing (TAP). The complete MHC class I heavy chain-β2m-peptide complex must be fully assembled before it can depart the ER and be delivered to the cell surface. In the absence of β2m or of peptide, MHC class I is retained in the ER and is unavailable for recognition by T cells.

For general information on the Major Histocompatibility Complex, see, for example, Srivastava et al., Immunogenetics of the Major Histocompatibility Complex, Vch Pub. (March, 1991); B. Pernis and H. J. Vogel, Cell Biology of the Major Histocompatibility Complex, Academic Press (October, 1995); T. W. Mak and J. Simard, Handbook of Immune Response Genes, Plenum Pub. Corp. (February, 1998); R. E. Humphreys and S. K. Pierce, Antigen Processing and Presentation, Academic Press (August, 1994); J. Klein and D. Klein, Molecular Evolution of the Major Histocompatibility Complex, NATO Asi Series, Series H, Cell Biology, Vol. 59, Springer Verlag (January, 1992); L. B. Schook and S. J. Lamont, The Major Histocompatibility Complex Region of Domestic Animal Species, CRC Series in Comparative Immunology, CRC Press (September, 1996); U.S. Patent Nos. 5,364,762, 5,639,458 and 5,734,023.

The .174 line of lymphoblastoid cells (hereinafter, 'the .174 cells') carries a mutation that eliminates the function of the TAP transporter (DeMars *et al.*, Mutations that impair a posttranscriptional step in expression of HLA-A and –B antigens, PNAS 82:8183-8187 (1985); Hughes E, Hammond C and Cresswell P, Mis-folded major histocompatibility complex class I heavy chains are translocated into the cytoplasm and degraded by the proteasome, PNAS 94:1896-1901 (1997)). Consequently, proteasome-processed peptides are not available for assembly with MHC class I molecules in these cells. As a result, most MHC class I molecules (with the exception of those which can assemble with signal sequence peptides) are retained in the ER.

An assay based on cytotoxic T lymphocyte (CTL)-mediated cell lysis is used to identify compounds which permit MHC class I molecules to be released from the ER and expressed at the surface of .174 cells. A line of .174 cells expressing a specific MHC class I allele will be prepared by standard cDNA transfection techniques. CTL's which recognize a specific antigenic peptide in association with this class I allele will also be prepared by standard techniques (Yap K and Ada G, Cytotoxic T cells specific for influenza virus-infected target cells, Immunology 32: 151-159 (1977)). The .174 cells will be aliquoted into the wells of a 96 well cell culture plate. Each well will receive a quantity of a compound to be tested, after which they will be incubated for 90 minutes at 37°C. The 96 well plates will be centrifuged to pellet the .174 cells, after which the cells will be

resuspended in normal media without any added test compound. The media will contain the specific antigenic peptide. After a further two hour incubation at 37°C, CTLs will be added to each well. Cell lysis will be measured using a standard automated fluorometric assay for T cell toxicity (Brenan M and Parish C. Automated fluorometric assay for T cell toxicity. J Immuno. Methods 112:121-131, 1988). Any well which has received a compound that permits the incompletely assembled MHC class I-β2M complex to depart the ER, reach the cell surface and bind the antigenic peptide present in the medium will be susceptible to CTL-mediated lysis. A duplicate 96 well assay plate will receive the same chemical compounds but will not receive CTL cells. Detection of cell lysis on this duplicate plate will identify compounds which lyse cells directly, rather than through the MHC-mediated pathway. This assay will permit rapid and reliable identification of compounds which permit the release of incompletely assembled or mis-folded proteins from the ER. Furthermore, the assay is designed to be employed in the high throughput screening of libraries consisting of natural products or of combinatorially synthesized chemicals.

**Immunodiagnostics/Immunoassays.** This group of techniques is used for the measurement of specific biochemical substances, commonly at low concentrations in complex mixtures such as biological fluids, that depend upon the specificity and high affinity shown by suitably prepared and selected antibodies for their complementary antigens. A substance to be measured must, of necessity, be antigenic - either an immunogenic macromolecule or a haptenic small molecule. To each sample a known, limited amount of specific antibody is added and the fraction of the antigen combining with it, often expressed as the bound:free ratio, is estimated, using as indicator a form of the antigen labeled with radioisotope (radioimmunoassay), fluorescent molecule (fluoroimmunoassay), stable free radical (spin immunoassay), enzyme (enzyme immunoassay), or other readily distinguishable label.

Antibodies can be labeled in various ways, including: enzyme-linked immunosorbent assay (ELISA); radioimmuno assay (RIA); fluorescent immunoassay (FIA); chemiluminescent immunoassay (CLIA); and labeling the antibody with colloidal gold particles (immunogold).

Common assay formats include the sandwhich assay, competitive or competition assay, latex agglutination assay, homogeneous assay, microtitre plate format and the microparticle-based assay.